Aggregation Pheromone in the Larvae of *Tipula simplex* Doane: Mode of Action and Site of Production[1]

(Diptera: Tipulidae)

by

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Tipula simplex Doane, the range crane fly, was first described in 1901, and most subsequent references to the animal concern the destructiveness of the larval stages during periodic but as yet unpredictable outbreaks. Doane (1908) described one such outbreak in which he counted densities as high as 43,400 larvae/m^2.

In nonoutbreak years, density measurements are exacerbated by clumped distribution in the eggs and late instar larvae. The eggs, first instar larvae and early second instar larvae are clumped where the eggs are laid, the late second instar larvae disperse through the grass, and the third and fourth instar larvae are aggregated under cowpads and other debris (Hartman and Hynes, 1977).

Hartman and Hynes (1977) indicated that light and moisture influence the behavior of the larvae, and postulated that these factors affected the distribution of third and fourth instar larvae. They also found that filter paper impregnated with *T. simplex* feces had many more larvae aggregated under it than did filter paper impregnated with water (Hartman and Hynes, 1977) or with extract of cowpads (unpublished data).

This paper reports on tests carried out to determine the source and mode of action of the aggregation pheromone.

Methods and Materials

Fourth instar larvae of *Tipula simplex* were collected from under year old cowpads in unirrigated pasture belonging to the Boston Land Company in Yokohl Valley, Tulare County, Calif. The larvae were placed in a covered plastic container with small pieces of cowpad to provide food and moisture. In the laboratory the larvae were placed at 4°C until used.

Two possible sources of the pheromones were collected for use in the experiments - feces, and a brown fluid exuded from the anus. Larvae were placed in compartments with screen bottoms and were covered with sheets

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of damp filter paper which fit closely into each individual compartment and lay directly on top of the larvae. The fecal material fell through the screen onto damp filter paper. The brown fluid, hereafter referred to as spots, was absorbed by the filter paper on top of the larvae. Ten animals were placed in each compartment and left 18 hours at 4°C.

The spot sheets were air dried and used for testing within six hours. The feces were ground into a powder and 0.1 g was spread on each 12.7-15.2 cm sheet of damp filter paper. The sheets were air dried and held for testing. Both spot and feces paper were cut to fit the apparatus to be used on a given day.

A T-maze was used to determine if the pheromone acted as an attractant. The long section of the apparatus was 30.5 cm long, 2.5 cm wide and 3.8 cm deep and was separated from the entry arm by a removeable panel. The entire maze was covered by a lid which helped to reduce air movement and to stabilize the moisture level in the maze. Temperature and illumination remained constant at 23°C and 0.76 lux. A larva was placed in the short entry arm and as soon as the larva entered one of the long arms its choice was recorded. Twenty control tests were run with water impregnated filter paper which covered the bottom of both arms as a test for bias in the maze or the larvae. Then three series of tests (10 larvae each) were conducted: (1) feces impregnated paper in the right arm, water impregnated paper in the left; (2) feces impregnated paper in the left arm, water impregnated paper in the right arm; (3) spot impregnated paper in the right arm, water impregnated paper in the left arm. Each animal was used in one replicate of one test and then discarded.

A second method of testing attractancy was devised, and used to test for attractancy of different concentrations of the feces. A circular glass container 4.8 cm in diameter with four side exit tubes 1 cm in diameter arranged at 90° angles was used. Papers were prepared with either water, 10^{-1}, or 10^{-2} dilution of feces in distilled water. For each test two arms contained water impregnated papers and two contained papers impregnated with the same dilution of pheromone. A larva was placed in the center of the container and when it entered one of the side arms the choice was recorded. Ten tests were run with each dilution; each larva was used only once and then discarded.

Tests for locomotory inhibition were carried out in a 12cm x 11cm box. In the bottom of the box we placed a piece of graph paper and covered it with a piece of filter paper. When the filter paper was dampened, the grid of the graph was visible. The light was kept constant at 0.76 lux and the temperature was monitored. Each larva was placed in the center of the box and every square that the larva touched for the next 30 minutes was recorded, including reentry into a previously touched square. Because a great deal of variation exists in the rate of movement among individual larvae, each larva was used for three tests (1) a control test with water dampened filter paper (2) one with feces impregnated filter paper (3) one with spot impregnated filter paper. Each larva was tested on three consecutive days.

We varied the order of the tests to determine if time of day or learning had an effect on the performance of any larva. Ten larvae were used in this experiment. The temperature in the box fluctuated between 22.8° and 23.9°C, so we ran an analysis of variance for the regression between movement and temperature.

To determine the site of pheromone production, four larvae were dissected, the digestive tract separated into (1) esophagus and proventriculus (2) gastric caeca (3) ventriculus (4) intestinal caecum and (5) intestine, and the organs and contents were homogenized in 2 ml of distilled water immediately before use. The terminology used for the organs of the digestive tract is that of Byers (1961) for *Dolichopeza,* a related genus.

Six filter paper strips were evenly spaced along the length of a 10x100 cm box on top of a layer of damp eccospheres (a chemically inert artificial medium which passes through a screen, 10 meshes/cm). The papers were separated by a 2mm gap to prevent the test substances from leaking from one paper to the next. The six papers were moistened with either water or one of the tissue extracts. Seven larvae were placed on each paper, the lid was put on, and the box was left in a dark room for 24 hours. The larvae were removed after their location had been recorded. This test was repeated a second time with the order of the papers changed.

To observe the histological details, we made serial sections of the digestive tract. Fourth instar larvae were preserved in Bouin's solution, dehydrated, embedded in paraplast, cut at 17 μm on an AO rotary microtome, stained in acid fuchsin and Mallory's triple and destained with 2% phosphomolybdic acid (Galigher and Kozloff, 1964).

Results

Attractancy

Using the initial preference of the twenty control larvae (no pheromone in the maze) a binomial test was made which indicated there was no bias in the maze or the larvae (60% into right arm, 40% into the left).

The two possible sources of pheromone and a combination of the two sources were analyzed statistically for attractancy. 58% of the larvae chose feces impregnated paper over water; 50% of the larvae chose spot impregnated paper over water. The results were nonsignificant.

In the tests with the glass cylinder, the larvae also showed no significant preferences for either concentration of feces (40% preferred feces at either concentration)

Locomotory Inhibition

An analysis of variance for the regression between temperature and movement gave $f = .291$ indicating no correlation between these 2 variables. A multiple group analysis of the data showed no significant difference between data collected in the morning or afternoon, and no difference between days and no significant interaction between the variables. The results of the paired-difference tests to compare the number of squares

Table 1. Rates of Locomotion in the Presence of Test Substances

Source	Mean # squares expt.	Mean # squares cont.	Mean diff.	X^2	Significance
Feces	15.5	70.9	55.4	5267.44	$p<.05$
Spots	44.8	77.6	31.7	3099.8	n.s.

a larva moved on the pheromone impregnated papers versus the control papers are shown in Table 1. The feces significantly reduced the movement of the larvae; the spots did not.

Site of Production

A comparison of the number of larvae which aggregated on the extract of each digestive organ was made using the Duncan multiple-range test (Steel and Torrie, 1960). A significantly higher number of larvae congregated on papers impregnated with extracts of gastric caeca and ventriculus than with any other homogenate or with water (Table 2).

The walls of the gastric caeca and ventriculus are composed of columnar cells. All other organs of the digestive tract are lined by cuboidal cells. All these organs except the gastric caeca and ventriculus had a chitinous layer between the cells and the lumen.

Discussion

Aggregation pheromones can act as attractants or as locomotory inhibitors. If a pheromone is an attractant, the larvae will be drawn to the area where other larvae are gathered, presumably in microhabitats suitable for growth. If the pheromone is a locomotory inhibitor, the animals will have to encounter conspecifics by other means, but once they do they will slow down, forming aggregations.

Fecal aggregation pheromones which act as locomotory inhibitors have been studied in the garden symphylan, *Scutigerella immaculata* (Reeve and Barry, in press) and the German cockroach *Blattella germanica* (Bell *et al.*, 1972; Burk and Bell, 1973). Both sets of experiments involved releasing numbers of animals simultaneously into an apparatus with pheromone present in one area. Our technique, in which each larva was tested alone, has the advantage of eliminating the possibility of any other form of communication among individuals and gives quantitative information about locomotory rates.

The fecal aggregation pheromone of *Tipula simplex* is produced in and/or

Table 2. Test for Pheromone Source[2]

	% aggregating
Gastric caeca	28 a
Ventriculus	28 a
Intestinal caecum	14.6 b
Intestine and Rectum	12.2 b
Water	9.8 b
Esophagus and proventriculus	7.3 b

[2]Duncan multiple-range test. Numbers followed by the same letter are not significantly different ($p < 05$).

stored by the gastric caeca and the ventriculus. Of the more than 250 pheromones known to be produced by insects, production in the digestive tract is a mechanism utilized by only a few (Jacobson, 1974), and the hindgut accounts for the majority of these (Fletcher, 1969; Blum and Wilson, 1964; Schneider and Rudinsky, 1969; Hangartner, 1969; Ishii and Kuwahara, 1968; Pitman and Vite, 1963). In only one other insect, *Periplaneta americana,* is the pheromone found to be present in the anterior portion of the midgut (Bodenstein, 1970).

Tipula simplex larvae demonstrate an orthohydrokinesis, and negative phototaxis (Hartman and Hynes, 1977). In the field, the larvae congregate under debris where it is darker and during dry weather moister than the soil. As the larvae move out of the moist environment, their kinesis insures that they move more rapidly, increasing the chance that they move into another moist environment. Negative phototaxis will keep them under debris in bright light, but will not be effective during dark nights. A locomotory inhibitor also will aid in maintaining the crane fly larvae in clumps in preferred microhabitats. A second advantage of a locomotory inhibitor as an aggregator is that as it decreases the locomotory rate, it decreases the energy requirements of the larvae. Therefore in the presence of the pheromone, larvae should be able to develop with less food intake. Low food requirements for each larva may explain why such large populations of *Tipula simplex* can survive in a limited space in outbreak years.

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SCIENTIFIC NOTE

Evidence indicating *Ammophila* (Hymenoptera:Sphecidae) as host of *Spintharosoma* (Hymenoptera:Chrysididae). — The genus *Spintharosoma* Zimmermann occurs in southwestern to western United States and in northern Mexico. It is found in the Mediterranean area, also. No host data have been recorded, but the related genus *Euchroeus* Latreille is known to attack *Podalonia* (Sphecidae:Sphecinae) in Europe (Molitor, A., 1935, Konowia 14:1-7) and in Mongolia (Tsuneki, K., 1947, Mushi 17:43-60).

In August, 1978 we watched numerous individuals of *Ammophila californica* Menke nesting in the sand dunes near Antioch, Contra Costa County, California. *Spintharosoma mesillae* (Cockerell) were seen attending five of the nests. In two cases both male and female chrysidids were present, facing the nest entrance at distances of 4 to 30 cm but most often between 6 and 10 cm. In three instances a female *mesillae* followed an *Ammophila* into the burrow and both remained for about 15 seconds. No *Spintharosoma* were seen on the dunes except at *Ammophila* nests, where as many as seven females attended a single burrow. On another occasion one of us (McLaughlin) made note of a similar occurrence on August 20, 1978 in Ventura County, California near Ojai. A female *Spintharosoma* (species A, undescribed) was seen near a nest while it was being provisioned by *Ammophila pruinosa* Cresson. As the *Ammophila* was engaged in cleaning the nest after deposition of a geometrid larva, she entered the burrow, followed closely by the chrysidid. They remained in the nest for about 15 seconds. A host-parasite relationship of *Ammophila* and *Spintharosoma* had previously been suspected after a female chrysidid (species A) was seen entering the burrow of an *Ammophila marshi* Menke at Sagehen Creek, Nevada County, California on June 23, 1976. This observation was made by David Poirier during an Entomology summer course conducted by Bohart. — R. M. BOHART and J.D. McLAUGHLIN, *Department of Entomology, University of California, Davis, 95616.*